

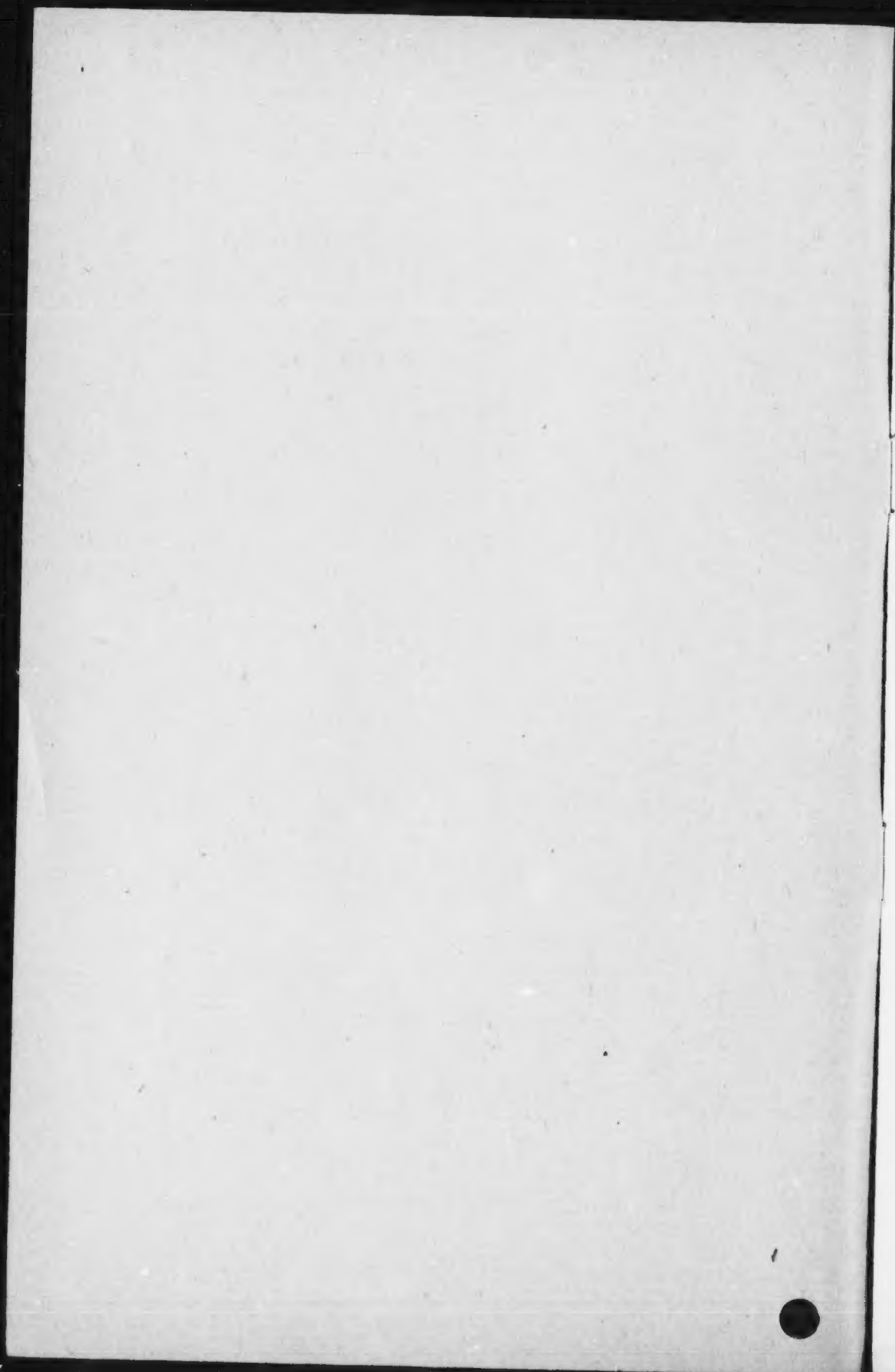


Studies in Liver Function

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In recognition of the need of better criteria for judging of the functional capacity of the liver in disease, this investigation was undertaken to determine (1) the degree and frequency with which functional changes can be demonstrated in anatomically diseased livers, (2) in what clinical types of liver injury the functional changes are most marked and (3) the diagnostic and prognostic value of these changes. In other words, an attempt has been made to regard the liver, in disease, from a physiologic point of view, and to study its various functions with the hope of ascertaining its behavior under pathologic conditions.

THE FUNCTION OF THE LIVER IN HEALTH

In health the liver has three well-established functions: 1. The glycogenic function, which has to do with carbohydrate metabolism, and consists in (*a*) the conversion of the monosaccharids (dextrose, levulose and galactose) into glycogen; (*b*) the temporary storage of that glycogen as such, and (*c*) the reconversion of it by enzyme activity into dextrose as needed in the body. 2. A participation in nitrogenous metabolism which consists in the conversion of certain nitrogenous bodies (ammonia, amino-acids, etc.), into urea. 3. The production of bile.

In addition, the liver apparently plays a part in the production of fibrinogen and antithrombin, although

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the connection of the latter functions with the liver is not so well established as the first three mentioned.

THE TESTS OF LIVER FUNCTION

The following tests based on well-known physiologic functions of the liver have been employed:

1. *Carbohydrate Metabolism*.—In this connection two tests have been employed, namely, Strauss'¹ levulose test and Bauer's² galactose test. The former consists in the ingestion of 100 gm. of levulose on an empty stomach and the examination of the urine voided during the next few hours to determine the presence or absence of the sugar, using Fehling's and Seliwanoff's tests. Bauer's is similar to Strauss' test except that 30 gm. of galactose are used instead of 100 gm. of levulose. The ingestion of these sugars in the amounts indicated does not result in their appearance in the urine in normal individuals, according to Strauss. Their appearance in the urine is due to a decrease in tolerance for these substances, indicative of decreased functional capacity of the liver.

2. *Nitrogenous Metabolism*.—In relation to nitrogenous metabolism and liver function a study of the nitrogen partition in the blood and in the urine has been made. If the liver converts the ammonia and amino-acids of the blood into urea, then it might be expected that in liver injury we should find a decrease in the urea percentage of the total non-protein nitrogen of the blood and an increase in the amino-acid nitrogen of the blood, while in the urine we might expect to find a decrease in the relative amount of urea with an increase in the ammonia and amino-acids. (The view that the liver is not wholly responsible for the conversion of the ammonia and amino-acids of the blood into urea has recently received support from the work of Folin³ and Van Slyke⁴ and of Fiske and Sumner.⁵)

3. *Urobilinogen*.—It has been maintained by many observers that the presence of urobilinogen in the urine is indicative of liver disease. Urobilinogen

1. Strauss: Berl. klin. Wehnschr., 1898, xxxv, 398; *ibid.*, 1899, xxxvi, 159; Deutsch. med. Wehnschr., 1901, xxvii, 757.

2. Bauer: Wien. med. Wehnschr., 1906, lvi, 20.

3. Folin: Jour. Biol. Chem., 1912, xi, 87.

4. Van Slyke, D. D.: Jour. Biol. Chem., 1913, xvi, 213; Protein Assimilation, abstr., THE JOURNAL A. M. A., April 12, 1913, p. 1183.

5. Fiske and Sumner: Jour. Biol. Chem., 1914, xviii, 285.

determinations were made in the majority of cases, but owing to the appearance of the recent comprehensive work of Wilbur and Addis,⁶ and to our own unsatisfactory results, these determinations were discontinued. These investigators showed conclusively that observations on a single or a twenty-four-hour specimen have no significance, since tremendous variations occur from day to day, and that only where repeated observations are made over a period of at least two weeks and then only when control studies of the urobilin content of the feces have been made to exclude blood destruction as a factor in the production of urobilinogen can findings be accepted.

4. *Fibrinogen*.—There is evidence to show that the liver is active in the formation of fibrinogen, and Whipple⁷ has suggested that the determination of the amount present in oxalated plasma may be used as an index of liver function.

5. *Lipase*.—Whipple⁸ has also suggested that the estimation of the lipolytic activity of the blood may constitute a test of liver function.

6. *Phenoltetrachlorphthalein*.—This drug was introduced by Rowntree, Hurwitz and Bloomfield⁹ as a test for hepatic function, and clinical studies by them and simultaneous experimental studies by Whipple and co-workers⁸ showed that a normal excretion could be established and that many liver injuries, particularly of severe grades, were associated with a decided decrease in the phenoltetrachlorphthalein output. Further studies with this test have been carried on.

7. *Fibrinolytic Ferment*.—Dr. Goodpasture of the pathologic staff was already interested in the presence of a fibrinolytic ferment in the blood in liver cirrhosis when this work was undertaken. Samples of blood from all our patients were submitted to him for study, and through his courtesy we present the results of his studies, details of which will appear later. The fer-

6. Wilbur, R. L., and Addis, Thomas: Urobilin: Its Clinical Significance, *Arch. Int. Med.*, February, 1913, p. 235.

7. Whipple and Hurwitz: *Jour. Exper. Med.*, 1911, xiii, 136. Whipple, G. H.: Hemorrhagic Disease—Septicemia, Melena Neonatorum and Hepatic Cirrhosis, *Arch. Int. Med.*, March, 1912, p. 365. Whipple, Mason and Peightal: *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 207.

8. Whipple, Mason and Peightal: *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 207. Whipple, Peightal and Clark: *Ibid.*, 1913, xxiv, 343. Whipple: *Am. Jour. Physiol.*, 1914, xxxiii, 50.

9. Rowntree, Hurwitz and Bloomfield: *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 327.

ment is specific for fibrin, does not attack fibrinogen and is destroyed by heating to 56 C. (132.8 F.).

METHODS AND NORMAL LIMITS OF TESTS

Phenoltetrachlorophthalein.—This test was employed according to the method described by Rowntree, Hurwitz and Bloomfield, details of which are given in full in their publication.⁹ The lower limit of normal excretion is considered to be 30 per cent. in forty-eight hours after the intravenous injection of 400 mg. of the substance. In health, phenoltetrachlorophthalein is practically never excreted in the urine after injection of such amounts.

Fibrinogen.—The heat coagulation method as described by Whipple¹⁰ was used in these determinations, and values below 350 gm. per hundred c.c. were regarded as abnormal.

Lipase.—Determinations of the lipolytic activity of the blood were made, using Loevenhart's¹¹ method, which consists in the determination of the total acid production of the blood when exposed to known quantities of ethyl butyrate at 37 C. (98.6 F.). The result is expressed as the number of cubic centimeters of tenth-normal acid produced by 1 c.c. of plasma and 0.26 c.c. ethyl butyrate, diluted to 4 c.c. and allowed to react for from twenty to twenty-four hours. The upper limit of normal has been taken to be 0.40 c.c. of tenth-normal acid.

The Nitrogen Partition in the Blood and Urine.—The total non-protein nitrogen of the blood was estimated by the micro-Kjeldahl method of Folin and Denis,¹² with slight modifications, as suggested by Dr. B. B. Turner.¹³ The urea percentage of the total non-protein nitrogen of the blood was determined by Marshall's¹⁴ method, utilizing soy bean urease, and values below 40 per cent. have been regarded as abnormal.

The amino-acid determinations were made by Van Slyke's¹⁵ method. As yet, data concerning the amount of amino-acids present in the blood of normal

10. Whipple: Am. Jour. Physiol., 1914, xxxiii, 50.

11. Loevenhart: Am. Jour. Physiol., 1902, vi, 331.

12. Folin and Denis: Jour. Biol. Chem., 1912, xi, 527.

13. These modifications will be described shortly by Dr. Turner.

14. Marshall: Jour. Biol. Chem., 1913, xv, 487.

15. Van Slyke: Jour. Biol. Chem., 1912, xii, 399.

individuals are scarce, but from a study of patients who were practically normal, 3 mg. per hundred c.c. of serum has been the value regarded as the upper limit of normal for the free amino-acid nitrogen.

The total nitrogen of the urine was determined on twenty-four-hour specimens collected under toluol by the Kjeldahl-Gunning method, the urea by Marshall's¹⁶ and the ammonia by Folin's¹⁷ method. The distribution of the urinary nitrogen between urea and ammonia has been shown to be dependent on the total excretion of nitrogen. The normal limits of the various nitrogenous constituents of the urine, with varying levels of nitrogen excretion, are therefore hard to define sharply. However, one can probably safely conclude that with a urinary nitrogen excretion of 10 gm. or more, the lower limit for urea nitrogen is 80 per cent. and the upper limit for ammonia nitrogen is 5 per cent., while with a nitrogen excretion of from 5 to 10 gm., the lower limit for urea is 70 per cent. and the upper limit for ammonia is 8 per cent. Furthermore, excessive acid production in the organism is accompanied with a lowering of the urea percentage and an increase in the ammonia percentage. The free amino-acid in the urine was determined by Van Slyke's¹⁸ method, and 1.5 per cent. has been adopted as the upper limit of normal. Future work may change these values.

SUMMARY OF FINDINGS

Forty-five cases have been studied in this series, in nearly all of which some abnormality of the liver was thought to be present. Some of the cases called cirrhosis were certainly not far advanced, and in some instances it appeared that the diagnosis was not definite, differences in opinion being frequently encountered. On account of lack of space the details of the cases are not given here,¹⁹ but a summary of the results obtained is given in Table 1.

Column 1 shows total number of tests made, Column 2 the total number of positive results, while the

16. Marshall: Jour. Biol. Chem., 1913, xiv, 283; xv, 495.

17. Folin: Ztschr. f. physiol. Chem., 1902, xxxvii, 161.

18. Van Slyke: Jour. Biol. Chem., 1913, xvi, 121.

19. Findings of all tests, clinical notes, necropsy findings in fatal cases together with a discussion of the functional findings appear in the article in the Proceedings of the Society of American Physicians, 1914.

remaining columns show the number of positive results in the various diseases. The figures in italics indicate the number of cases in which each particular test was applied in the particular disease studied, while the other figures indicate the number of positive findings in the particular disease.

From this table it will be seen that the following results were obtained: The *phenoltetrachlorphthalein* test was applied in the feces in 42 cases with 16, and in the urine in 34 cases with 21 positive. *Fibrinogen* was determined in 40 instances, 8 of which were below 350 mg. and 12 below 400 mg., while 8 were above 800 mg., that is, 20 abnormal findings. The *lipolytic activity* of the blood was investigated in 39 cases, 5 of which showed an acid production of more than 0.4 c.c. and 3 above 0.5 c.c. The presence of *fibrinolytic ferment* was tested for in 40 instances, and found in 7, all of which were cirrhoses. The *galactose* test was applied in 29 and the *levulose* in 19 cases, the former showing only 4 and the latter 2 positive findings. The *total non-protein nitrogen* and *urea nitrogen of the blood* were determined in 37 cases. The urea nitrogen was less than 40 per cent. of the total in 9 instances. The *amino nitrogen of the blood* was determined in 35 cases, and amounted to more than 3 mg. per hundred c.c. in 25 instances and above 4 mg. in 13 instances. The *total nitrogen urea and ammonia in the urine* were determined in 41 cases. The total nitrogen of the urine was above 10 gm. in 13 cases, and in these the urea nitrogen was below 80 per cent. in 7 instances, and the ammonia nitrogen above 5 per cent. in 10 instances. Twenty-one cases showed a urinary nitrogen between 5 and 10 gm., and of these, 4 cases showed a urea nitrogen below 70 per cent. and 11 an ammonia nitrogen above 8 per cent. The *amino-acid nitrogen* content of the urine was investigated in 22 cases, 12 of which showed values above 1.5 per cent. of the total nitrogen.

Liver Cirrhosis.—The results in the fourteen cases of cirrhosis studied were as follows: Phenoltetrachlorphthalein studied in feces in 14, positive in 6, in urine in 11, positive in 7; fibrinogen in 14, positive in 6; lipase in 14, positive in 2; fibrinolytic ferment in 14, positive in 7; galactose in 11, positive in 2; levulose in 10, positive in 2; urea nitrogen percentage (blood) in

TABLE 1.—SUMMARY OF FINDINGS

Number of cases tested	Total Number of Tests	Total Number of Positive Tests	Cirrhosis of Liver	Myocardial Insufficiency	Carcinoma	Syphilitic Hepatitis	Pernicious Anemia	Polyserositis	Miscellaneous	
Phthalen in feces	42	16	14	9	3	2	2	2	9	10
Phthalen in urine	34	21	11	10	3	1	2	0	9	4
Fibrinogen ..	36	8	14	11	3	2	1	2	5	4
Fibrinolytic ferment ..	40	7	14	12	1	0	—	2	1	1
Lipase	39	5	14	12	1	0	—	0	9	0
Galactose	29	4	11	7	1	0	—	0	9	0
Levulose	18	2	10	3	0	1	1	0	4	2
Blood: Urea-N % ..	37	9	14	12	1	—	1	0	3	0
NH ₂ -N %	35	25	14	9	1	2	—	2	6	0
Urine: Urea-N % ..	41	11	14	11	3	2	2	1	6	4
NH ₂ -N % ..	41	28	14	11	2	2	2	0	8	2
NH ₂ -N % ..	22	12	7	6	1	1	1	1	5	2

14, positive in 5; amino nitrogen (blood) in 14, positive in 10; urea percentage (urine) in 14, positive in 3; ammonia percentage (urine) in 14, positive in 10; amino nitrogen percentage (urine) in 7, positive in 5.

Myocardial Insufficiency.—Twelve cases of myocardial insufficiency were studied. Phenoltetrachlorphthalein studied in feces in 9 cases with 1 positive, lowest being 18 per cent., and in urine 10 cases with 5 positives; fibrinogen in 11 with 1 positive; lipase in 11 cases with 1 positive; fibrinolytic ferment in 12, positive in none; galactose in 7, positive in none; levulose in 3, positive in none; urea nitrogen percentage (blood) in 12, positive in 1, in which 6 were over 50 per cent., and 3 over 60 per cent.; amino nitrogen (blood) in 9, positive in 7.

The functional injury in liver cirrhosis is relatively greater than in chronic passive congestion, for a consideration of all the tests in each condition indicates that in 10 of the 14 cases of cirrhosis there was definite functional injury, 3 with less marked injury and only one with a practically normal function throughout, while in chronic passive congestion only 4 showed definite decrease, while 7 showed a practically normal function.

Carcinoma of Liver.—Little data outside of the phenoltetrachlorphthalein have been collected. C 28, who definitely had metastasis to the liver,²⁰ showed a 23 per cent. output five months before death. H 29, with gastric carcinoma and clinically thought to have metastasis to the liver, showed a 24 per cent. output, phenoltetrachlorphthalein positive in urine and the urea only 21 per cent. of the total non-protein nitrogen of blood. These cases are in keeping with the three cases of liver carcinoma reported last year in which the phenoltetrachlorphthalein excretion was 8, 14 and 7 per cent., respectively. However, a slight decrease in phenoltetrachlorphthalein, for instance, from 20 to 25 per cent. does not indicate liver carcinoma, since such findings have been found in gastric carcinoma when no proof of liver metastasis was at hand, in which case it is merely the expression of the influence of anemia and cachexia on liver function.

²⁰ Details of these cases can be found in the article in the Proceedings of American Physicians.

Anemia.—Clinically, a severe grade of anemia depresses the function of the liver as indicated by phenoltetrachlorphthalein. That there is also a decrease in the fibrinogen has been shown by Whipple, but owing to the quantity of blood necessary for carrying out the test, it is not applicable in all cases of anemia. Changes in the urinary nitrogen partition may appear.

Lytic Hepatitis.—Of the two cases studied, one showed normal phenoltetrachlorphthalein, a low lipase, urinary nitrogen partition changes and a positive galactose, while the other showed a low phenoltetrachlorphthalein content in feces, positive in urine, an abnormally high fibrinogen, low urea percentage of total non-protein nitrogen in blood and urinary nitrogen partition changes.

Polyscrosisitis.—Two cases with much the same clinical condition and both with large livers showed practically identical findings, for instance, normal phenoltetrachlorphthalein in feces, but positive in urine, fibrinogen and lipase normal, low urea percentage of total non-protein nitrogen in blood, and changes in urinary nitrogen partition, but no decreased tolerance for sugar.

THE PHENOLTETRACHLORPHTHALEIN TEST

Previously the results of eighty applications of the test on sixty-seven patients were reported. The total number of patients studied to date is 113, thirty-three of whom had no clinical evidence of liver disease. The positive findings in health and in various types of liver disease, together with the appearance of the drug in the urine, are shown in Table 2.

NECROPSY CASES

In this series five cases came to necropsy, and pathologic findings controverting the functional findings have not been encountered. It is of interest to note that two of the cases which clinically were thought to be cirrhosis of the liver, but showed slight functional changes, proved, at necropsy, to have no cirrhosis at all, but to be cases of chronic peritonitis in which the liver was enlarged owing to a thickened capsule. In one instance, a case of cirrhosis, microscopic study of the liver suggested greater injury than was shown by the functional studies. In the six cases which came to necropsy last year there was harmony between the

anatomic and functional findings of the phenoltetrachlorophthalein test.

RELATIVE VALUES OF TESTS

The series of cases is not sufficiently large to draw far-reaching conclusions as to the relative values of the different tests, but the following impressions have been received:

(a) Phenoltetrachlorophthalein: A phenoltetrachlorophthalein output in the feces below 30 per cent. or its appearance in the urine is infrequent in health and frequent in anatomically diseased livers. A normal output does not exclude liver injury.

(b) Fibrinogen: Low values are frequent in cirrhosis, but inconstant. Very low findings may carry prognostic significance.

TABLE 2.—POSITIVE FINDINGS IN HEALTH AND IN LIVER DISEASE

	Examined	Less Than 30 Per Cent.	Urine Examined	Positive
Normals	33	2	22	3
Cirrhosis	18	9	11	7
Congestion ..	32	11	10	6
Carcinoma of liver	6	6	3	2
Carcinoma ...	5	3	3	2
Luetic liver..	5	2	2	1
Severe anemia	9	7	1	0
Miscellaneous.	5	2	2	2
Totals	113	42	54	23

(c) Lipase: Determination of lipolytic activity of blood furnishes little or no information of diagnostic or prognostic value in these types of cases.

(d) Bauer's galactose and Strauss' levulose tests yielded very little information in this series.

(e) Dr. Goodpasture's fibrinolytic ferment studies on this series of cases show that this ferment is present only in cirrhosis and hence when present is of diagnostic value.

(f) Blood Nitrogen Partition: Urea nitrogen was low in several instances and especially low in cases of advanced cirrhosis. Amino-acid nitrogen was high in a considerable proportion of the cases.

(g) Urinary Nitrogen Partition: The ammonia and aminonitrogen were increased in most of the

cases studied, particularly in cirrhosis. Owing to the non-exclusion of acidosis considerable difficulty is experienced in interpreting these results. It would have been advisable to have attempted to exclude acidosis by the feeding of alkalies.

PRACTICABILITY OF TESTS

The phenoltetrachlorophthalein test involves an intravenous injection and careful collection of stools for forty-eight hours. Three undesirable features are connected with its application: 1. The quality of red color obtained in certain instances (from 10 to 20 per cent. of the cases) is such that accurate quantitative estimation is difficult. Only in rare instances is this a serious difficulty leading to error of more than 5 per cent. 2. Thrombosis frequently follows at the point of injection, as evidenced by slight local pain or tenderness together with resistance offered to the palpating finger along the course of the vein. This occurred in eight instances in this series of cases, but in no case has it occasioned serious discomfort or resulted in actual harm to the patient. 3. With salt solution made with freshly distilled water chills following the injection are infrequent. When no dilution is employed chills are less frequent and not accompanied with increase in temperature.

Fibrinogen determinations are inapplicable in the presence of severe anemia. They necessitate the withdrawal of from 40 to 50 c.c. of blood. A high speed centrifuge and considerable chemical equipment are requisites. The test is time-consuming. *Lipase determinations* require neither complicated apparatus nor much time. *The determination of the total non-protein nitrogen, urea nitrogen and amino-acid nitrogen* of the blood cannot be carried out when severe anemia exists. Technically the determinations are easy but are time-consuming. *The nitrogen partition* in the urine requires somewhat less time. *Galactose* can be administered without discomfort to the patient, but technical difficulties attend the use of *levulose*, at least 25 per cent. of the patients refusing to take the full amount or developing nausea or vomiting following its ingestion. *The fibrinolytic ferment* is readily demonstrated. *Urobilinogen determinations* in the urine are valueless unless made daily for a period of two weeks and unless associated with urobilin determinations in the feces.

CONCLUSIONS

1. Outspoken changes in liver function can be demonstrated in most cases of advanced liver cirrhosis, in markedly congested livers associated with myocardial insufficiency, in carcinoma of the liver, in luetic livers and in conditions of cachexia with marked anemia.

2. Functional changes have been most marked in cirrhosis, neoplasm of the liver and in cachectic conditions associated with severe grades of anemia. Functional changes in chronic passive congestion have not been frequent or pronounced.

3. Harmony in the findings of the tests is present in some cases, that is, most of the tests indicating either a decrease function or a normal function. However, lack of harmony is striking in other cases.

4. Concerning the relative merits of these tests the impressions that have been received are that the determination of the phenoltetrachlorophthalein excretion, the fibrinogen, and the nitrogen partition in the blood and urine are of decided value in revealing the presence of, and to a less degree the extent of, functional involvement, while the demonstration of a fibrinolytic ferment is of decided diagnostic importance. The determination of the sugar tolerance and of the lipolytic activity of the blood apparently afford information of much less value.

The information derived from these studies on the liver does not compare in diagnostic and prognostic value with that derived from corresponding studies of the kidney. This may depend on several factors, namely, (a) the inadequacy of our knowledge of liver physiology; (b) the limited number of cases and of types of severe liver injury under observation; (c) the possible existence of a great "factor of safety" in the liver, whereby in case of necessity function is carried on efficiently by a relatively small proportion of liver substance; (d) the fact that the prognosis in many of these cases is controlled by factors other than decreased liver function, and (e) the lack at present of correlation of anatomic, clinical and functional findings occasioned by the newness of the subject. The results, however, encourage us in the belief that scientifically and also clinically this subject is worthy of extensive investigation.

